Helios User Manual

Contact info:

Software login Username: administrator (no password)

Transfer/storage of data

- ✓ Files on E-drive will be deleted without warning!
- ✓ Export your data (fcs-files). From the CyTOF computer you can export data via:
    1. E-mail
    2. Dropbox/GoogleDrive etc.
    3. Your private Cytobank account
- ✓ Always cut your folder from E-drive and paste to external hard drive Seagate expansion (F-drive) when you are done running your samples.

You might experience trouble with the software if the E-drive is more than 25 % full!!

Booking and Payment

Booking: Check the booking system for availability and to **request** a booking time (icr-booking.no), *Flow cytometry Montebello* schedule ("Helios/Hyperion"). The core facility staff will approve or deny your request, as the staff needs to be available for startup of the instrument. If your request is denied, try booking another time, or send an e-mail to flowcytometri@rr-research.no.

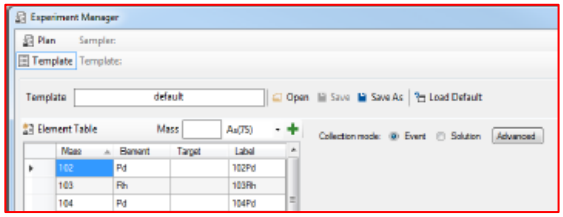
Runtime invoicing: The runtime of your booking will be invoiced as a general rule, therefore it's important to show up on time, and predict your runtime as precisely as possible when booking.
Any changes to the runtime that you weren't able to edit must be e-mailed to us, so we can change the booking length. In the case of instrument malfunction, we will of course not charge the user. Contact us if there are other circumstances (out of your control) prolonging/delaying your run.

Reagent purchases: If you purchase reagents from the core facility, it must be entered in the spreadsheet *"CyTOF reagents"* on the desktop on the CyTOF workstation in room K03-078 (same room as the fridge with antibodies/conjugation kits). The file has separate sheets for antibodies, conjugation kits and barcodes.

The EQ-beads contains:

Cerium (Ce)	140	Europium (Eu)	151	Holmium (Ho)	165	Lutetium (Lu)	175
Cerium (Ce)	142	Europium (Eu)	153			Lutetium (Lu)	176

Running samples

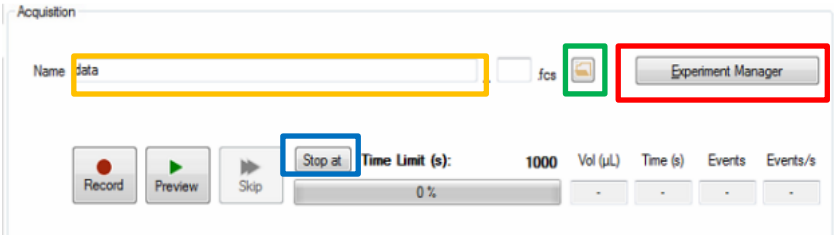


Samples in 5 ml tubes for the PSI (pressurized sample introduction).

Before running the first sample:

Open the *Acquire* tab. Press the **folder icon**, and make a folder with date and name under E: "All runs stored here" and select that folder. Close the folder window. Press **Experiment Manager** and find/update the template you wish to use (located on E:/Templates).

Remember to include in your template:



- All EQ bead channels, as they are needed for normalization (140Ce, 142Ce, 151Eu, 153Eu, 165Ho, 175Lu and 176Lu)
- Background channels: 80ArAr, 120Sn, 131Xe, 132Xe, 138Ba, 133Cs, 208Pb, 127I
- All metal channels for your antibody stains and other relevant channels (Barcode channels, Cisplatin, IdU ++)
- Iridium (191Ir and 193Ir)

Close the experiment manager window when your template is open/selected. Use collection mode: *Event*. Make sure *Preserve IMD* and *Gaussian Discrimination* is checked under advanced. Press *ON* under sample introduction to start running DIW. Click *Preview* to check the background and *Stop* when background is ok. **Always wait for the stable blue light before opening the PSI.**

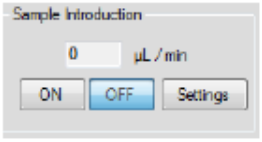
Running samples

1) Prepare your sample (resuspend in CAS containing 10% EQ beads, filter the sample). **Write file name**. Take out CAS tube from PSI to insert sample tube.

2) Adjust the ***Stop at*** value (NB: tube dead volume: 50µL). Press *Preview*. Your sample will start entering the detector after 30s(flow rate:30µL/min), and the stable event rate will show after 60-70 seconds. Optimal event rate is 350evts/sec. Dilute until event rate is OK, Press *Record*. When recording has finished or you wish to stop recording, press *Stop*.

3) During recording, pay attention to the sample introduction rate (should be 30 µl/min) and the PSI pressure (should be stable, and between 10-15). To check the PSI pressure, open **Status Panel > Sample Introduction >** Pressure. If pressure is unstable and high, it's most likely buildup of a clog. Contact the core facility staff for assistance, or see *"Troubleshooting PSI Helios"* section in this guide.

4) In between samples, run 3 minutes with CAS by loading a CAS tube in the PSI and press *ON* under *Sample Introduction* in the lower part of the software. Press *Preview* to check the background, and *Stop* when background looks ok. You can run Wash solution to improve background and minimize carry-over. Remember to always run CAS after wash solution (double the time of the wash-run)!



5) Go back to 1) to run the next sample.

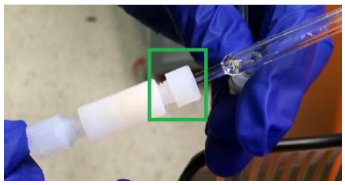
After your last sample: Cleaning and Shutdown

- **Log in to the booking site** (icr-booking.no) to check if there is a user running after you. If so, click on their booking to view their phone number, and contact them to make sure they will show up. If not reachable, contact the core facility staff. If the next user is coming, wash the sample line (next step), and leave the instrument with a DIW tube docked on the PSI and sample introduction OFF. If you are the last user, perform all the cleaning/shutdown steps below.

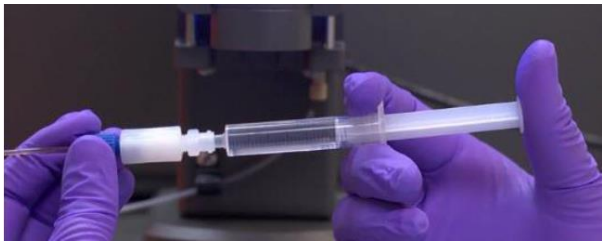
- **Wash sample line**: Insert a tube with Wash solution to the PSI and press *ON* by sample introduction. Wash for 5 minutes. Press *OFF*, then insert a tube with DIW. Press *ON* and let the DIW run for 5 minutes. Press *Preview* to check the background. When the background is ok (if not ok, check with *Preview* every 30 s until ok), press *OFF*. Use *Snipping Tool* to snapshot the Rain Plot and store the image in the Background Folder as "PostRunBackgroundYYMMDD"

- **Turn off plasma:** Press the big green button to turn off Plasma. Wait until the *Plasma Stop Sequence has been completed successfully*-message appears.

- **Turn off the Argon gas** (on the wall).



- **Clean nebulizer:** Loosen the beige nebulizer port half a turn to the left, to remove the nebulizer. Remove the sample capillary from the nebulizer by turning the connector (image) closest to the nebulizer half a turn then gently pulling out the capillary. Then disconnect the nebulizer from the gas line. Using the Nebulizer Cleaning Kit in the cupboard, slowly draw 10% Decon 90 through the sidearm and sample inlet of the nebulizer and soak for 15 min. Rinse the nebulizer 2 to 3 times with DIW using the same kit. Leave the nebulizer submerged in a DIW bath. Put all boxes back in the cupboard and place lids on top.



- **Backflush PSI sample line:** Take out the DIW tube and insert an empty tube while leaving the PSI lid open and depressurized. Unscrew the blue fitting connected to the capillary, take up DIW in the cleaning syringe, mount the cleaning syringe to the blue fitting and press DIW through the PSI line until 10 drops of water have gone into the empty tube. Reinsert the DIW tube (min. volume: 1 mL) into the PSI and close the lid. "Park" blue fitting in the left side of the PSI where there is a hole the fitting screws into.

- **Clean WB injector:** Use heat protection gloves to take off the heat shield above the heater assembly. **Take off the clamp** that connects the heater to the WB injector. Gently pull out the heater-assembly and place on pins above. Carefully take out the WB injector and wash through it with DIW 2-3 times and leave on a KimWipe in its cardboard box (in the cupboard).

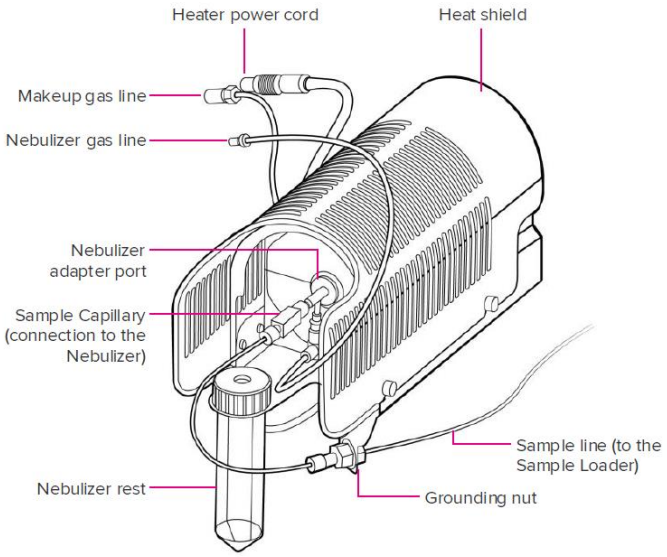
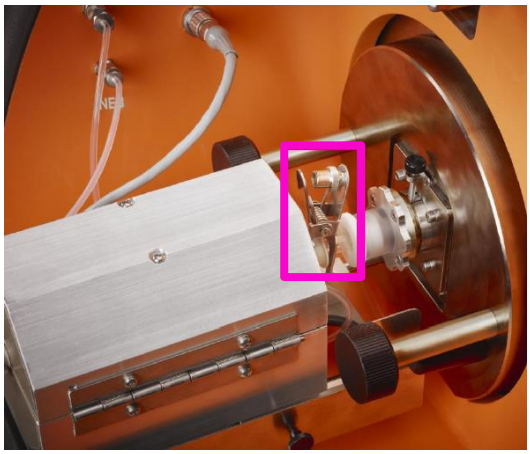
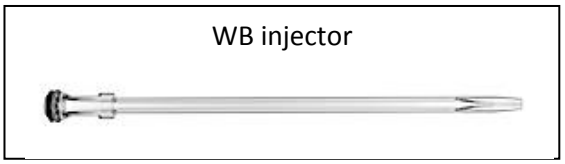


Figure 19. Heater assembly and connections to the Sample Loader





- **Data processing: Normalization/bead removal/concatenating** (optional; can be done on stand-alone computer later) FCS Processing window is found in the "Process" tab of the software.
- **Export your data (fcs-files).** From the CyTOF computer you can export data via E-mail, Dropbox/GoogleDrive etc., or your private Cytobank account (no USBs allowed). If you are the last user of the day, cut your folder from E-drive and paste to external hard drive Seagate expansion (F-drive).
- **Remember to note if you have purchased any antibodies, conjugation kits or barcodes in the "CyTOF reagents" excel file on the analysis computer in room K03-078.**

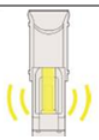
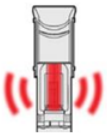
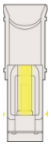
Analysis: We have a CyTOF workstation located in room K03-078 that can be used for data analysis in FlowJo, or FCS processing in the CyTOF software (normalization, concatenation, debarcoding) if this wasn't done on the CyTOF computer after acquisition

PSI/Sample Loader LED Indicator Colors

The Sample Loader has three LED colors with flashing function to indicate the operational status of the module. When the LED indicator is white, the handle is in the open position and the Sample Loader is ready for sample loading.

When a sample of cells, beads, or tuning solution has been loaded into the 5 mL tube and the Sample Loader handle is pushed down and closed, the LED indicator turns to blue.
Go to **Sample Introduction** and click **ON** in the CyTOF software and the module begins to pressurize until the LED indicators begins to glow blue. This means that the system is pressurized and ready to begin tuning the system or to preview or to record your data in the CyTOF software. The 5 mL round-bottom tube containing the sample will be agitated and sample will be taken up into the nebulizer.
NOTE To check the PSI pressure, open **Status Panel > Sample Introduction >** Pressure. The pressure will tell you if the instrument is clogging.

IMPORTANT Do not open the handle of the PSI when the LED indicator is glowing blue and pressurized. This may cause the sample to become aerosolized. In this case the LED indicator will quickly flash yellow to indicate an error and the system will immediately begin depressurizing.

LED Indicator Color	Description
White (stable) 	Handle is open. Ready for loading sample.
Blue (stable) 	The sample is loaded and handle is closed. The PSI is ready to pressurize .
Glowing Blue 	The system is pressurized and sample is being delivered to the nebulizer. CAUTION: Do NOT open the handle!
Flashing Yellow 	The system is depressurizing. CAUTION: Do NOT open the handle!
Flashing Red 	The PSI agitator has a fault detected. See Troubleshooting in the Helios manual for more details.
Yellow (stable) 	The PSI is depressurized, but likely there is a clog. If the sample introduction rate is 0 and PSI pressure is 0, open handle and close again to return to stable blue color, before troubleshooting the clog.

Troubleshooting PSI Helios

NOTE To check the pressure in the Sample Loader, open **Status Panel > Sample Introduction >** Pressure.
Always try to use the WASH solution first if suspecting a clog (NB: due to the viscosity the wash solution always has higher psi values than the ones stated below. When troubleshooting the location of the clog, use milliQ water!

1. If everything is connected (PSI through nebulizer), your Pressure should be **10-15 psi**, definitely below 20 psi. As you start forming clogs, the pressure will start creeping up. Also, your event rate will start to fluctuate.

2. If everything apart from the nebulizer is connected (PSI through nebulizer capillary), your Pressure should also be **10-15 psi**, definitely below 20 psi.

3. If PSI and sample line are connected with the blue fitting to the grounding nut (but nebulizer and nebulizer capillary are not connected), your pressure should be **below 4 psi**.

4. If PSI and sample line are connected but sample line blue fitting is NOT connected to the grounding nut, your pressure should also be **below 4 psi**.

5. If PSI is connected but sample line is DISCONNECTED from the bottom of the PSI, your pressure should be **1.5-2.5 psi** (definitely below 3 psi).

If your Pressure values differ from the above, or if the PSI automatically starts blinking yellow when you start running water or sample, you probably have a clog, and can use the above values to help isolate where the clog is. For example, your pressure with all lines but no nebulizer (#2) is 25 psi, but your pressure without the nebulizer capillary (#3) is 3psi, the clog is probably in the nebulizer capillary and it should be replaced. More nebulizers are soaked in water in the top shelf of the CyTOF wash cupboard. Alternative capillary lines are either placed by the instrument or in plastic bags marked “Montert” (in a box in the lower shelf in the cupboard above and next to the workstation). On the desktop, the file “nebulizers and capillaries” has an overview of which capillaries are good matches with which nebulizers.

The Core facility staff would like to help you with Helios clogging issues, but if we are not available send us an email about the location of clogged capillaries or nebulizers.

The main reasons for Helios clogging are:

- Sample quality (e.g. Dissociated tissue vs PBMC, cell stickiness and sedimentation)
- Sample preparation (clumping during fixation etc.)
- Too high cell concentration in samples (perfect event rate is 350 events/s)

Take steps to test possible improvements of your sample prep if your samples are consistently clogging the instrument. We would like to hear what works for your samples, so as to better help the Helios users ☺